In the Specification:

Please amend the specification as shown:

Please delete paragraph [0013] and replace it with the following paragraph:

[0013] Figure 1A illustrates the structure of HPMA copolymer-Gly-Phe-Leu-Gly **(SEQ ID NO: 1)** -ethylenediamine-TNP-470. Figure 1B shows *in vitro* release of TNP-470 from HPMA copolymer in the presence (-■-) and absence ((-♦-) of cathepsin B.

Please delete paragraph [0014] and replace it with the following paragraph:

[0014] Figure 2A shows inhibition of BCE proliferation *in vitro* after 72 h. TNP-470 (-▲-) and HPMA copolymer- Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-en-TNP-470 (-■-) had similar cytostatic effect on bFGF-induced proliferation of endothelial cells at doses lower than 1 μg/ml and cytotoxic effect at doses higher than 1 μg/ml. The dotted line represents the proliferation of bFGF-induced BCE cells (- - -) and the solid line represents the BCE cell proliferation in the absence of bFGF (⁻). Figure 2B shows the chick aortic ring endothelial sprouting assay. The effect of TNP-470 (central panel) and HPMA copolymer- Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-en-TNP-470 (right panel) at 100 pg/ml TNP-470 equivalent-dose are shown; and a control chick aortic ring (left panel) with abundant sprouting.

Please delete paragraph [0016] and replace it with the following paragraph:

[0016] Figure 4 shows antitumour activity measured using male SCID mice bearing A2058 human melanoma. Figure 4A shows the effect of TNP-470 (-●-); HPMA copolymer- Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-en-TNP-470 (-▲-); and control mice (-■-) on tumors. Data represent mean ±SE, n=8 mice per group. P values of <0.05 were marked as *, P<0.03 **, P<0.01 ***. Figure 4B shows SCID mice and excised tumors correlating to panel (A) at day 8 of treatment. Figure 4C shows H & E staining of tumors excised from animals in different groups on day 8 at high and low power.

Please delete paragraph [0017] and replace it with the following paragraph:

[0017] Figure 5 shows antitumour activity measured using male C57 mice bearing LLC. Figure 5A shows the effect of TNP-470 at 30 mg/kg/q.o.d. s.c. (-●-); HPMA copolymer- Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-en-TNP-470 at 30 mg/kg/q.o.d. s.c. (-▲-) on tumor growth; control mice (-■-) are also shown. Data represent mean ±SE, n=10 mice per group. Figure 5B shows representative C57 mice correlating to (A) on day 10 following treatment. Figure 5C shows dose escalation of HPMA copolymer- Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-en-TNP-470: at 30 (-▲-), at 60 (-●-) and at 90 mg/kg/q.o.d. (-♦-) and control mice (-■-) are shown. Data are mean ± SE, n=10 mice per group. Figure 5D shows C57 mice correlating to (C). P values of <0.05 were marked as *, P<0.03 as **, P<0.01 as ***.

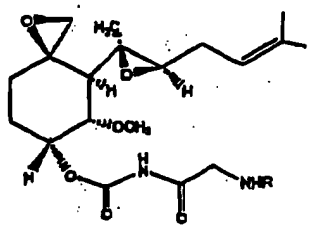
Please delete paragraph [0019] and replace it with the following paragraph:

[0019] Figure 7 shows the effect of TNP-470 on serum-induced cell proliferation. Inhibition of BCE (open symbols) and A2058 (closed symbols) cell proliferation *in vitro* after 72 h. TNP-470 (-●-) and HPMA copolymer-GFLG **(SEQ ID NO: 1)**-en-TNP-470 (-▲-) had similar cytostatic effect on bFGF-induced proliferation of endothelial cells at doses lower than 1 μg/ml and cytotoxic effect at doses higher than 1 μg/ml. The dotted line represents the proliferation of bFGF-induced BCE or serum-induced A2058 cells (—) and the solid line represents the BCE and A2058 cell proliferation in the absence of bFGF or serum, respectively (- - -).

Please delete paragraph [0025] and replace it with the following paragraph {**NOTE**: please keep original formula inserted within the paragraph}:

[0025] Cleavage of the linker of the conjugate results in release of active TNP-470. Thus the TNP-470 must be conjugated with the polymer in a way that does not alter the activity of the agent. The linker preferably comprises at least one cleavable peptide bond. Preferably the linker is an enzyme cleavable oligopeptide group preferably comprising sufficient amino acid units to allow specific binding and cleavage by a selected cellular enzyme. Preferably the linker is at least two amino acids long, more preferably at least three amino acids long. For example, TNP-470 can be conjugated to HPMA

copolymer-Gly-Phe-Leu-Gly (SEQ ID NO: 1) -ethylendiamine via nucleophilic attack on the α-carbonyl on the TNP-470 releasing the chlorine to form a compound of formula 1,



wherein R is $(CH_2)_nR'$, where n is 0 to 3, preferably n is 2, and R' is NH_2, O or S. For instance, HPMA copolymer-Gly-Phe-Leu-Gly (SEQ ID NO: 1)-ethylendiamine (100 mg) can be dissolved in DMF (1.0 ml). Then, TNP-470 (100 mg) can be dissolved in 1.0 ml DMF and added to the solution. The mixture is stirred in the dark at 4 ^{0}C for 12 h. DMF is then evaporated and the product, HPMA copolymer-TNP-470 conjugate is redissolved in water, dialyzed (10 kDa MWCO) against water to exclude free TNP-470 and other low molecular weight contaminants, lyophilized and stored at –20 ^{0}C. Reverse phase HPLC analysis using a C18 column, is used to characterize the conjugate. This conjugated structure can be cleaved enzymatically between the glycine residue of the peptide and the ethylenediamine residue (See Figure 1A).

Please delete paragraph [0036] and replace it with the following paragraph:

[0036] In a most preferred embodiment, L is a Gly-Phe-Leu-Gly-(SEQ ID NO: 1) linkage. In one embodiment, U is an ONp group, wherein Np is a p-nitrophenyl group. Preferably y is in the range 0.3 to 15 and x is in the range of 99.7 to 85. Most preferably, y is in the range of 5-10 and x is in the range of 90-95. In a more preferred embodiment, the polymeric backbone is HPMA copolymer- Gly-Phe-Leu-Gly (SEQ ID NO: 1)-ethylenediamine having the values for x and y as defined above.

Please delete paragraph [0047] and replace it with the following paragraph:

[0047] A random copolymer of HPMA copolymerized with methacryloyl-Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-*p*-nitrophenyl ester (HPMA copolymer-MA-GFLG **(SEQ ID NO: 1)** -ONp) incorporating approximately 10 mol% of the MA-GFLG **(SEQ ID NO: 1)**-ONp monomer units was prepared as previously reported [24] and provided by Polymer Laboratories (UK). The polymeric precursor was used for ethylenediamine (en) incorporation and the product HPMA copolymer-GFLG **(SEQ ID NO: 1)**-en had a Mw of 31,600 Da and polydispersity (PD) of 1.66. TNP-470 was kindly provided by Douglas Figg from the NCI (USA). 2-Propanol, methanol, orthophosphoric acid and chloroform were from Sigma (all HPLC grade). Dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were from Aldrich (USA). All other chemicals were of analytical grade from Aldrich (USA) and Fisher Chemicals (USA) unless otherwise stated. Vivacell 70 ml (10 kDa MW cut-off PES) was from VivaScience (USA). Isoflurane was purchased from Baxter Healthcare Corporation (USA). Matrigel basement membrane matrix (from Engelbreth-Holm-Swarm mouse tumor) was purchased from Becton Dickinson (USA). Avertin was purchased from Fisher (USA).

Please delete paragraph [0049] and replace it with the following paragraph:

[0049] TNP-470 was conjugated to HPMA copolymer-Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-ethylendiamine via nucleophilic attack on the α-carbonyl on the TNP-470 releasing the chlorine. HPMA copolymer-Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-ethylendiamine (100 mg) was dissolved in DMF (1.0 ml). Then, TNP-470 (100 mg) was dissolved in 1.0 ml DMF and added to the solution. The mixture was stirred in the dark at 4 ^{0}C for 12 h. DMF was evaporated and the product, HPMA copolymer-TNP-470 conjugate was redissolved in water, dialyzed (10 kDa MWCO) against water to exclude free TNP-470 and other low molecular weight contaminants, lyophilized and stored at –20 ^{0}C. Reverse phase HPLC analysis using a C18 column, was used to characterize the conjugate.

Please delete paragraph [0056] and replace it with the following paragraph:

[0056] HPMA copolymer-Gly-Phe-Leu-Gly **(SEQ ID NO: 1)**-ethylenediamine-TNP-470 conjugate (Fig. 1A) was synthesized, purified and characterized by HPLC. Gly-Phe-Leu-Gly **(SEQ ID NO: 1)** polymer-TNP-470 linker was designed to permit intralysosomal TNP-470 liberation due to action of the lysosomal

cysteine proteases[29], such as cathepsin B. It has been shown that cathepsin B is overexpressed in many tumor cells[30]. The conjugate accumulates selectively in the tumor tissue due to the EPR effect and is slowly internalized into endothelial cells in the tumor bed by fluid-phase pinocytosis. The conjugate should not internalize into normal quiescent endothelial cells, hence will not be exposed to lysosomal enzymes leaving the linker intact. Free TNP-470 eluted as a single peak with a retention time of 13.0 min while the conjugate eluted as a wider peak at 10.0 min (results not shown). Free drug was negligible (<0.01% of total TNP-470) following repeated purification by dialysis. TNP-470 is not water-soluble but became soluble following conjugation with HPMA copolymer. The conjugate was stable for three days in phosphate buffered saline or citrate buffer, pH 5.5, 0.2 M at 37 ^{0}C. However, under the same conditions with the addition of the lysosomal enzyme cathepsin B, the linker between the polymer and the drug (Gly-Phe-Leu-Gly[31]) **(SEQ ID NO: 1)** was cleaved and TNP-470 was released (Fig. 1B). These conditions imitate the lysosomal environment in endothelial cells where lysosomal enzymes, such as cathepsin B, are present. TNP-470 release from the conjugate reached a plateau within 5 h of incubation with cathepsin B and did not increase appreciably even after 5 days. The incubated solution was then analyzed and had a TNP-470 content of approximately 10 mol%. We next tested the HPMA copolymer-TNP-470 conjugate activity in two *in vitro* angiogenesis assays: the endothelial cell proliferation and the chick aortic ring assays.

Please delete paragraph [0066] and replace it with the following paragraph:

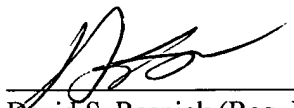
[0066] Polymer-angiogenesis inhibitor conjugates can capitalize on the ability of macromolecules to target solid tumor tissue passively by the EPR effect[26] (similar to PK1). This effect occurs due to the poorly organized tumor vasculature[41] resulting in 'enhanced permeability' towards circulating molecules. The poor lymphatic drainage in tumor tissue leads to increased 'retention'. It is accepted that the main reason for the improved antitumor activity of the polymer-drug conjugates, with respect to the free drug, is tumor targeting as a result of this EPR effect[37]. Gly-Phe-Leu-Gly **(SEQ ID NO: 1)** polymer-TNP-470 linker used in this study was designed to permit intralysosomal TNP-470 liberation due to action of the lysosomal cysteine proteases[29]. In order to exert an antitumor effect, an active TNP-470 species must be released at the tumor site and interact with methionine aminopeptidase 2 (MetAP2) in endothelial cells. MetAP2 is one molecular target of TNP-470 that was recently identified, although the precise mechanism underlying its selective effect on the proliferation of endothelial cells is yet to be understood[42].

Therefore, the T/C values for the conjugate of 0.12 - 0.14 indicated that TNP-470, which was bound to the polymeric backbone during circulation, was released at the tumor site. The mechanism for release of a TNP-470 moiety involves cellular uptake, followed by enzymatic cleavage of the peptide linker within the lysosomes of endothelial cells. It is likely that some of the conjugate that accumulates in the tumor will be taken up by tumor cells. However, a higher concentration of TNP-470 will be needed to affect tumor cells (3-logs higher).

The Commissioner is hereby authorized to charge any additional fees associated with this communication to Deposit Account No. 50-0850.

Date: July 20, 2004

Respectfully submitted,



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